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(54) Title: NOVEL BAKER'S YEAST AND DOUGHS CONTAINING THE SAME

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(57) Abstract: A baker's yeast which exhibits a strong fermentation power in doughs with a high osmotic pressure containing sodium chloride and various baking materials in baking methods such as the straight method, the sponge dough method and/or the freezing method and thus enables the production of favorable bread products having a large specific volume. More particularly speaking, a baker's yeast which is tolerant to a high osmotic pressure and withstands freezing in baking methods such as the straight method, the sponge dough method and/or the freezing method.

(57) 要約:

本発明は、パン製法のストレート法、中種法および／または冷凍法において、食塩および種々の製パン材料を加えた高浸透圧生地での発酵力が強く、比容積が大きく良好なパンを製造し得るパン酵母を提供する。詳細には、パン製法のストレート法および／または中種法において耐浸透圧性を有し、且つ冷凍耐性を有するパン酵母が提供される。

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ABSTRACT

The present invention provides baker's yeast which has strong fermenting ability in dough with a high osmotic pressure containing salt and various breadmaking ingredients in breadmaking methods, i.e., a straight dough method, a sponge dough method and/or a freezing method, and enables production of good bread having a large specific volume. More particularly, the present invention provides baker's yeast which is tolerant to osmotic pressure and freezing in breadmaking methods, i.e., the straight dough method and/or the sponge dough method.

DESCRIPTION

NOVEL BAKER'S YEAST AND DOUGH CONTAINING THE SAME

TECHNICAL FIELD

The present invention relates to novel baker's yeast and a method for breadmaking using the yeast. More particularly, the present invention relates to baker's yeast which is tolerant to osmotic pressure in fermentation of bread dough and a method for breadmaking with a straight method, a sponge dough method, and a freezing method using dough containing various breadmaking ingredients as well as the baker's yeast.

BACKGROUND ART

Some bread is made of dough containing no or a small amount of sucrose added (e.g., French bread, white bread, etc.), and other bread is made of dough containing a large amount of sucrose added (e.g., sweet bread, etc.). Bread is thus made of various types of dough. Baker's yeasts having different levels of fermenting ability are used in breadmaking, depending on the amount of sugar. For bread dough containing a large amount of added sucrose, baker's yeast having high sugar tolerance has been selected.

Sugar tolerance conventionally means an ability to tolerate sucrose. The sucrose tolerance of baker's yeast has been long studied and there have been reports on the relationship with the activity of invertase. Invertase is an extracellular enzyme that degrades sucrose which is a disaccharide of the two monosaccharides, glucose and

fructose. After sucrose is extracellularly degraded by invertase into monosaccharides, the monosaccharides are taken into a yeast body and utilized as a nutrient. In the case of baker's yeast having a high level of invertase activity, degradation of sucrose into monosaccharides is accelerated, so that osmotic pressure around baker's yeast in dough is increased and suppresses fermentation by baker's yeast. Thus, it is suggested that there is a negative correlation between invertase activity and sucrose tolerance. Actually, baker's yeast which is currently used for sweet bread is selected from strains having a low level of invertase activity (Technical Report of The Japan Yeast Industry Association, 58, 77 (1988)).

Further, there were reports showing that sucrose tolerance was increased by actively breeding baker's yeast having a low level of invertase activity. Examples of such baker's yeast include: one that has a low level of invertase activity, is also freeze tolerant, and can be used in breadmaking using dough containing 25% sucrose (Japanese Laid-Open Publication No. 7-203952); one that has a low level of invertase activity, and has a high level of ability to ferment even in dough containing 30% sucrose (Japanese Laid-Open Publication No. 8-154666); and one that has a low level of invertase activity and a high level of maltase activity, and can be used in producing both white bread and sweet bread (Japanese Laid-Open Publication No. 9-149785). Thus, it is indicated that the invertase activity is involved in an ability to ferment in dough containing a high concentration of sucrose. As described above, however, the baker's yeast having a low level of invertase activity is not tolerant to a high sucrose concentration per se, but decelerates degradation of sucrose into its monosaccharide

components (i.e., glucose and fructose) suppressing an increase in osmotic pressure around the baker's yeast, resulting in sucrose tolerance.

There is a report indicating that in fermentation of sweet bread dough, sucrose tolerance is less affected by involvement of invertase activity than the osmotolerance of baker's yeast (Food Microbiol, 7, 241 (1990)). As an example showing involvement of osmotolerance in sucrose tolerance, there is a report indicating that the fermenting ability of baker's yeast in dough having a relatively high sucrose concentration is strongly correlated with the amount of glycerol in the baker's yeast body (Appl. Environ. Microbiol, 63, 145 (1997)) and another report indicating that glycerol was externally added and taken into baker's yeast bodies, so that the fermenting ability of the baker's yeast is improved (Food Microbiol, 15, 51 (1998)). Further, as an example showing that sucrose tolerance is improved, there is a report indicating that the osmotolerance of baker's yeast could be improved by adding inorganic salt, such as NaCl, KCl, etc. into a culture of baker's yeast, thereby enhancing the fermenting ability of the baker's yeast in sweet bread dough (US Patent No. 4,420,563 (1983)).

Thus, the examples which have been reported on sucrose tolerance indicate that invertase activity is involved in sucrose tolerance, and that the involvement of invertase is limited. In other words, sucrose tolerance is a combined property of invertase activity and osmotolerance. To improve sucrose tolerance, most reports have intended to breed strains having a low level of invertase activity. In these cases, sucrose tolerance along with a practical fermenting ability level has been exhibited only when the

sucrose content was 30% or less.

There are two representative methods for breadmaking. One is a straight dough method in which the fermenting ability of baker's yeast is immediately reflected. In this method, all ingredients for bread dough are mixed at one time and allowed to ferment, followed by baking. The other is a sponge dough method in which bread dough is made in two steps. In this method, pre-fermented dough (i.e., sponge dough) is made and fermented at first, another dough is made and mixed with the sponge dough, and the resultant mixture (herein referred to as a final dough) is made and allowed to ferment. The sponge dough method is often used in breadmaking since the method has the advantages of an increase in bread volume due to the flexibility and gas retaining ability of the bread, an improvement in the machine tolerance of bread dough, etc.

As an example of preparation of baker's yeast suitable for these two breadmaking methods, there is a report indicating yeast having sucrose tolerance in the straight dough method and the sponge dough method (Japanese Laid-Open Publication No. 10-191964). In this example, sucrose tolerance was exhibited in dough containing 30% sucrose in the straight dough method and in final dough containing 25% sucrose in the sponge dough method.

Further, baker's yeast having freeze tolerance as well as sucrose tolerance has been developed. There are the following examples of the baker's yeast having freeze tolerance and sucrose tolerance: IAM4274 (Japanese Laid-Open Publication No. 59-203442) having an excellent final proof after freezing and specific volume in sweet bread dough

(sucrose 25%); FTY-2 (Japanese Laid-Open Publication No. 7-203952) having freeze tolerance (it is described that the amount of gas produced in dough containing 30% sucrose was slightly less than when commercially available baker's yeast was used); baker's yeast (US Patent No. 4,547,374) having a satisfactory result in a freeze preservation test of dough having 25% sucrose; baker's yeast (Japanese Laid-Open Publication No. 7-203952) having a low level of invertase activity and freeze tolerance, and able to be used in breadmaking using dough containing 25% sucrose; baker's yeast (Japanese Laid-Open Publication No. 8-154666) having fermenting ability after thawing at a residual rate of at least 90% in dough containing 30% sucrose. Further, based on the idea that the intracellular trehalose content is involved in freeze tolerance, the NTH or ATH gene encoding an enzyme degrading the trehalose was destroyed by a recombinant technique to suppress degradation of trehalose in order to improve freeze tolerance (Japanese Laid-Open Publication No. 10-117771 and Japanese Laid-Open Publication No. 11-169180).

In most of the above-described examples, freeze tolerance was obtained in sweet bread containing 25% sucrose with respect to flour. The greatest amount of sucrose added was 30%.

(Problem to be Solved by the Invention)

The breadmaking industry often uses not only sucrose but also isomerized liquid sugar, which is converted from a part of glucose to fructose by isomerase treatment. In sucrose-added dough, as sucrose is degraded to monosaccharides by the invertase of baker's yeast, osmotic pressure is gradually increased. In isomerized sugar-added

dough containing glucose and fructose, and sucrose-mixed and isomerized sugar-added dough, baker's yeast ferments a dough having a high level of osmotic pressure from the beginning.

Further, salt and other sub-ingredients for breadmaking other than sugars are added to bread dough so as to improve flavor and taste, thereby producing distinctive bread. Representative examples of the bread sub-ingredients mixed to bread dough other than sugars include fats, dairy products such as milk, skim milk powder, etc., and eggs. Some of these bread sub-ingredients added to bread dough affect osmotic pressure similarly to salt. Therefore, the coexistence of such a bread sub-ingredient and sugar or salt increases osmotic pressure, whereby the fermenting ability of baker's yeast is suppressed.

As described above, in the conventional straight dough method and freezing method, examples of baker's yeast exhibited sucrose tolerance when the sucrose content of dough was 30% or less. In the conventional sponge dough method, there were only reports that sucrose tolerance was exhibited in final dough containing 25% sucrose. In dough containing at least 30% of sucrose, or dough containing 30% or less of sugar but further containing isomerized sugar, and high osmotic pressure dough containing salt or bread sub-ingredients so that osmotic pressure is increased, fermentation is suppressed, thereby making it more difficult to obtain a sufficient volume of bread. There was no known baker's yeast which is suitable for fermentation in high osmotic pressure dough. The fermentation of known baker's yeast was insufficient in any of the straight dough method, the sponge dough method and the freezing method for

breadmaking.

The objective of the present invention is to prepare novel osmotolerant baker's yeast capable of strong fermentation in high osmotic pressure dough which is conventionally difficult, and novel baker's yeast which can be used in the straight dough method, the sponge dough method and the freezing method for breadmaking in various high osmotic pressure dough.

DISCLOSURE OF THE INVENTION

(Means for Solving the Problem)

Strains were isolated from nature and were repeatedly subjected to screening for osmotolerance, sponge dough tolerance and freeze tolerance. The present invention has succeeded in preparing practical strains having excellent levels of these functions.

In one aspect, the present invention relates to baker's yeast having osmotolerance in fermentation of bread dough.

In one embodiment, the present invention relates to baker's yeast having the above-described properties in the straight dough method. In another embodiment, the present invention relates to baker's yeast having the above-described properties in the final fermentation of the sponge dough method. In a still another embodiment, the present invention relates to baker's yeast having the above-described properties in final dough, both in the straight dough method and the sponge dough method. The present invention also relates to baker's yeast having freeze tolerance.

In one embodiment, as to the baker's yeast of the present invention, the amount of carbon dioxide gas generated at 38°C for 2 hours is at least 140 ml for every 50 g of isomerized sugar containing dough, corresponding to 35% of sugar.

In another embodiment, the present invention relates to bread dough containing the baker's yeast of the present invention. In still another embodiment, the present invention relates to a method for breadmaking using the baker's yeast of the present invention.

BEST MODE FOR CARRYING OUT THE INVENTION

Hereinafter, the present invention will be described in detail.

First, the terms as used herein will be described below. The terms as used herein have the same meanings as those usually used in the art, except for the terms particularly specified below.

In the present specification, the percentage (%) of sucrose, salt, and other bread sub-ingredients refers to percentage by weight with respect to flour. For example, 30% sucrose means that 30 g of sucrose is used with respect to 100 g of flour. The term "bread sub-ingredient" as used herein refers to ingredients which may be used in breadmaking, other than flour, salt and water. Examples of the bread sub-ingredients include, but are not limited to, sucrose, isomerized sugar, dairy products, eggs, fats.

The term "high osmotic pressure dough" as used herein refers to dough having the number of moles corresponding to at least 30% of sucrose with respect to flour where the number of moles is calculated by summing the number of moles of sucrose (e.g., sucrose, isomerized sugar, etc.), salt, and ingredients involved in osmotic pressure which are contained in dairy products or the like, in breadmaking ingredient. The term "osmotolerant baker's yeast" as used herein refers to baker's yeast having strong fermenting ability in high osmotic pressure dough containing breadmaking ingredients (e.g., sucrose, glucose, fructose, sucrose-mixed isomerized sugar, isomerized sugar, salt, dairy products, etc.). Further, the term "strong fermenting ability" refers to, for example, that the amount of carbon dioxide gas generated at 38°C for 2 hours is at least 140 ml in every 50 g of isomerized sugar containing dough, corresponding to 35% of sugar.

The term "isomerized sugar" as used herein refers to sugar containing glucose and fructose which sugar is obtained by converting a part of glucose with isomerase. A mixture of glucose and fructose can also be included in isomerized sugar. The term "isomerized sugar containing dough" as used herein refers to dough containing glucose and fructose. An example of such dough is, but not limited to, one that contains 50% glucose and 50% fructose. Further, in the bread-making industry, "sucrose-mixed isomerized sugar" containing sucrose is often used as isomerized sugar. The term "sucrose-mixed isomerized sugar containing dough" as used herein refers to dough containing sucrose, glucose and fructose. For example, the sucrose-mixed isomerized sugar containing dough is, but not limited to, dough containing 50% sucrose, 25% glucose and 25% fructose.

The baker's yeast of the present invention having osmotolerance in bread dough fermentation is tolerant to high osmotic pressure in bread dough made of various breadmaking ingredients. For example, such tolerance includes, but is not limited to, glucose tolerance, fructose tolerance, isomerized sugar tolerance, salt tolerance, dairy product tolerance, etc.

The baker's yeast of the present invention having excellent osmotolerance exhibits sucrose tolerance even in sucrose-containing dough containing at least 30% sucrose. Conventionally bred baker's yeast strains having sucrose tolerance due to a reduction in invertase activity acquire sucrose tolerance by suppressing an increase in osmotic pressure around the baker's yeast due to degradation of sucrose. Thus, it is clear that such conventionally bred baker's yeast is different from the osmotolerant baker's yeast of the present invention.

In one embodiment, the osmotolerant baker's yeast of the present invention is characterized in that the amount of carbon dioxide gas generated at 38°C for 2 hours is at least 140 ml, more preferably at least 150 ml, and even more preferably at least 160 ml, in every 50 g of isomerized sugar containing dough, corresponding to 35% of sugar. More preferably, in addition to the amount of carbon dioxide gas generated in isomerized sugar containing dough of 35% sugar concentration, the baker's yeast of the present invention is characterized in that the amount of carbon dioxide gas generated at 38°C for 2 hours is at least 180 ml, preferably at least 190 ml, and more preferably at least 200 ml, in dough containing 40 g of sucrose with respect to 100 g of

flour.

In one aspect, the baker's yeast of the present invention may have sponge dough tolerance. The term "sponge dough tolerance" refers to osmotolerance in the final dough fermentation subsequent to fermentation of sponge dough.

Usually, in the sponge dough method, dough having a low sugar concentration (about 3%) is prepared at first and is allowed to ferment (referred to as first fermentation or sponge dough fermentation). It is believed that this sponge dough fermentation allows the baker's yeast to be active. In final fermentation after the sponge dough fermentation, high osmotic pressure is created due to added sugar ingredients and other breadmaking ingredients. The active baker's yeast is suddenly allowed to ferment in high osmotic pressure dough. Therefore, baker's yeast having osmotolerance after activation due to sponge dough fermentation (i.e., first fermentation) is suitable for the sponge dough method. Such baker's yeast is referred to as sponge dough tolerant baker's yeast.

The baker's yeast of the present invention exhibits strong fermenting ability and remains osmotolerant in final fermentation after sponge dough fermentation in spite of the presence of a high concentration of added sucrose, isomerized sugar, and breadmaking ingredients, such as salt, etc. which increase the osmotic pressure of dough. Specifically, the sponge dough tolerant baker's yeast of the present invention which is tolerant to osmotic pressure in final fermentation after sponge dough fermentation is characterized in that the amount of carbon dioxide gas generated at 38°C for 2 hours is at least 140 ml, more

preferably at least 150 ml, in every 50 g of final dough whose composition is shown in Table 1 after sponge dough fermentation at 30°C for 150 minutes.

As described above, the baker's yeast of the present invention exhibits osmotolerance in any of the straight dough method and the sponge dough method.

Table 1

Ingredients		
	Sponge dough	Final dough
Flour	70 g	30 g
Yeast	3 g	-
Glucose	3 g	15 g
Fructose	-	15 g
Water	39 ml	15 ml
Steps		
1) Mixing sponge dough		
2) Sponge dough fermentation at 30°C for 2.5 hours		
3) Mixing final dough		
4) The amount of gas generated in every 50 g of dough was measured with a Fermograph (made by ATTO Co.) at 38°C for 2 hours. The resultant value is regarded as the amount of gas generated in final dough.		

In another aspect, the baker's yeast of the present invention is also characterized by freeze tolerance in high osmotic pressure dough. Conventionally, there was only a report that freeze tolerance was obtained in dough having 30% or less of sucrose. There was no example reported that has freeze tolerance when containing over 30% of sucrose, or in high osmotic pressure dough containing isomerized sugar, salt, or a dairy product, etc.

The fermenting ability before freezing are important

for the fermenting ability after freezing as well as the strength of freeze tolerance. The baker's yeast of the present invention has strong freeze tolerance, and strong fermenting ability in high osmotic pressure dough before freezing, so that the strong fermenting ability is maintained after freezing. The fermenting ability after freezing of the baker's yeast of the present invention is represented by the amount of carbon dioxide gas generated in every 50 g of dough which is mixed and is allowed to ferment at 30°C for 60 minutes, followed by freeze preservation for a given time, and after thawing at 25°C for 60 minutes, is allowed to ferment at 38°C for 2 hours. The osmotolerant and freeze-tolerant baker's yeast of the present invention is characterized in that the amount of carbon dioxide gas generated in 35%-sucrose dough after freezing for 4 weeks is at least 300 ml, preferably at least 320 ml, and that the amount of carbon dioxide gas generated in dough containing 30% of sucrose-mixed isomerized sugar and 1% salt after freezing for 4 weeks is at least 270 ml, preferably at least 280 ml.

The present invention relates to baker's yeast having osmotolerance in the straight dough method. Further, the present invention also relates to sponge dough tolerant baker's yeast having osmotolerance in final dough fermentation in the sponge dough method. Preferably, the present invention relates to baker's yeast having osmotolerance both in the straight dough method and a sponge dough method. Further, preferably, the present invention relates to baker's yeast having osmotolerance in the straight dough method and the sponge dough method and having freeze tolerance.

The present invention relates to bread dough containing flour and breadmaking ingredients (e.g., sugars, salt, eggs, fats, dairy products, emulsifiers, etc.), and the baker's yeast of the present invention. The baker's yeast of the present invention can be used in various bread dough ranging from low sugar concentration to high sugar concentration. Particularly, the baker's yeast of the present invention is suitable for dough having high osmotic pressure due to the presence of sugars and various breadmaking ingredients. The baker's yeast of the present invention is suitable for any of the straight dough method and the sponge dough method, and can be sufficiently used after freeze preservation.

The baker's yeast of the present invention is not particularly limited as long as it has osmotolerance in the straight dough method and/or the sponge dough method for breadmaking, and has freeze tolerance. The baker's yeast of the present invention can be obtained from nature by screening and obtained by a breeding technique for baker's yeast, such as crossing, mutation, cell fusion, etc. Preferably, the baker's yeast of the present invention can be prepared by cross breeding of a plurality of strains including a strain having freeze tolerance. Crossed strains are screened to obtain osmotolerant and sponge dough tolerant strains, and are further screened to obtain freeze tolerant strains. From these strains, baker's yeast can be prepared by a culture method shown in the Examples.

A preferable baker's yeast of the present invention is Saccharomyces cerevisiae. A representative example of a crossed strain selected by the above-described method is the KKK47 strain, which is a strain of Saccharomyces

cerevisiae. The KKK47 strain is deposited at the National Institute of Bioscience and Human-Technology, the Agency of Industrial Science and Technology, the Ministry of International Trade and Industry (1-1-3 Higashi, Tsukuba, Ibaraki, Japan) under the depository number FERM BP-7267 on August 31, 1999 (receipt of request for deposit dated August 7, 2000).

Examples

Hereinafter, examples of the present invention will be described. These examples are only intended for illustrative purposes. The present invention is not limited to the examples. Ingredients used in the examples are the following: flour is Camellia manufactured by Nisshin Flour Milling; yeast food is Kaneplus C (manufactured by Kaneka Corporation); shortening is Snow Light (manufactured by Kaneka Corporation); and margarine is Nova 11 (manufactured by Kaneka Corporation). Other breadmaking ingredients and bread sub-ingredients are available from general stores. As control strains, three baker's yeast strains commercially available from Kaneka Corporation were used.

Commercially available baker's yeast A
(regular yeast manufactured by Kaneka)

Commercially available baker's yeast B
(freeze tolerant yeast manufactured by Kaneka)

Commercially available baker's yeast C
(freeze tolerant yeast manufactured by Kaneka)

Example 1: Crossed breeding

Among Saccharomyces cerevisiae stock strains processed by the Applicant three strains, including two strains having freeze tolerance, were used as original strains. All of these original strains were diploid. The original strains were allowed to form spores in sporulation medium, followed by cross breeding.

1) Spores derived from the two freeze tolerant strains were crossed with spores derived from a common yeast strain, thereby preparing a number of first-generation crossed strains.

2) The first-generation crossed strains were allowed to form spores. The spores derived from the different original freeze tolerant strains were crossed with each other, thereby preparing second-generation crossed strains.

3) The second-generation crossed strains were allowed to form spores. Crossing was conducted in various combinations, thereby preparing third-generation crossed strains.

A number of crossed strains of each generation were screened for osmotolerance, sponge dough tolerance and freeze tolerance, thereby obtaining parent strains for next-generation crossed strains. An attempt was made to improve these capabilities for each generation. The KKK47 strain of the present invention having the intended capabilities was eventually acquired in the third-generation crossed strains.

Example 2: A method for producing baker's yeast

Medium having a composition shown in Table 2 was

prepared as volumes of 5 ml/large test tubes and 50ml/500ml Sakaguchi flasks, which were in turn autoclaved. The resultant medium was used.

A platinum loop of the cross-bred strain was inoculated into the large test tube, followed by shaking culture at 30°C for one day. The resultant culture was transferred to the 500 ml Sakaguchi flask, followed by shaking culture at 30°C for one day. The resultant yeast was subjected to 5L jar culture.

Table 2

Flask seed yeast culture

Medium composition	
Sugar (molasses)	4.0%
Urea	0.3%
Ammonium sulfate	0.08%
Potassium dihydrogen-phosphate	0.04%
Zinc sulfate	5 ppm

2L of medium having a composition shown in Table 3 was poured into a 5L jar, and was autoclaved. The yeast collected from five 500 ml Sakaguchi flasks was inoculated and cultured under the conditions shown in Table 4.

Table 3

5L jar seed yeast culture

Medium composition	
Sugar (molasses)	90 g
Urea	6.75 g
Ammonium sulfate	1.8 g
Potassium dihydrogen-phosphate	0.9 g
Zinc sulfate	11.25 mg
Water	2250 ml

Table 4

Airlation volume	2.0 nl/min
Agitation	650 rpm
Temperature	33°C
pH	4.7 control (using 14% ammonium water)

· 5L jar main culture

50 g of seed yeast bodies cultured in the 5L jar was added as wet yeast bodies to starting liquid having a medium composition shown in Table 5, and cultured under the conditions shown in Table 6.

Table 5

5L jar main culture

Medium composition	
Sugar (molasses)	230 g
Urea	4.9 g
75% phosphate	1.4 ml
Zinc sulfate	20 mg
Copper sulfate	3.15 mg
Vitamin B1	10.5 mg
Water	2000 L

Table 6

Airlation volume	2.5 nl/min
Agitation	650 rpm
Temperature	33°C
pH	4.7 control (using 14% ammonia water)

Culture was conducted for 13 hours. Sugars were added portion by portion during 12-hour culture.

The cultured yeast was centrifuged immediately after

the culture, and were suctioned and dehydrated with a suction funnel, thereby preparing wet yeast bodies. The wet yeast body was used in the examples below. When used in an experiment, the moisture content of the wet yeast was measured, and the amount used was calculated based on 65% moisture.

The amount of gas generated was compared between the thus-obtained baker's yeast of the present invention and the commercially available strains using the straight dough method (Examples 3 to 7). The amount of carbon dioxide gas generated was measured as follows: dough ingredients described in each example were mixed with a Hobart desk-top mixer for 3 minutes, the amount of gas generated in every 50 g of the dough was measured at 38°C for 2 hours with Fermograph (manufactured by ATTO).

Example 3: Straight dough method - Osmotolerance (1)

The amounts of carbon dioxide gas generated in glucose containing dough and fructose containing dough having a composition shown in Table 7 were measured and compared between the KKK47 strain, and commercially available baker's yeasts A, B and C. The results are shown in Table 8.

Table 7

Dough composition

	Glucose dough	Fructose dough
Flour	100 g	100 g
Baker's yeast	4 g	4 g
Glucose	35 g	-
Fructose	-	35 g
Water	50 ml	50 ml

Table 8

Amount of gas generated

	Glucose dough	Fructose dough
KKK47	231 ml	163 ml
Commercially available baker's yeast A	151 ml	86 ml
Commercially available baker's yeast B	160 ml	90 ml
Commercially available baker's yeast C	186 ml	115 ml

Fermenting ability in high osmotic pressure dough containing 35% sugars (glucose and fructose) with respect to flour was compared between the KKK47 strain of the present invention, and the commercially available baker's yeasts to find that the KKK47 strain generated a larger amount of gas than the commercial yeasts. Thus, the KKK47 strain is considered to have superior osmotolerance.

<u>Example 4: Straight dough method - Osmotolerance (2)</u>

The amounts of carbon dioxide gas generated in sucrose-mixed isomerized sugar containing dough and isomerized sugar containing dough (sugar concentration: 35%) having compositions shown in Table 9 were measured and compared between the KKK47 strain, and commercially available baker's yeasts A, B and C. The results are shown in Table 10.

Table 9

Dough composition

	Sucrose-mixed isomerized sugar containing dough	Isomerized sugar containing dough
Flour	100 g	100 g
Baker's yeast	4 g	4 g
Sucrose	17.5 g	-
Glucose	8.75 g	17.5 g
Fructose	8.75 g	17.5 g
Water	50 ml	50 ml

Table 10

Amount of gas generated

	Sucrose-mixed isomerized sugar containing dough	Isomerized sugar containing dough
KKK47	239 ml	187 ml
Commercially available baker's yeast A	163 ml	116 ml
Commercially available baker's yeast B	172 ml	116 ml
Commercially available baker's yeast C	211 ml	135 ml

The KKK47 strain generated a larger amount of gas compared to the commercially available yeasts. In this case, the sugar concentration was 35%. The sucrose-mixed isomerized sugar containing dough and the isomerized sugar containing dough had higher osmotic pressure than sucrose containing dough having the same sugar concentration. Thus, the KKK47 strain is considered to have superior osmotolerance in sucrose-mixed isomerized sugar containing dough and

isomerized sugar containing dough, as found in the results for glucose containing dough and fructose containing dough.

Example 5: Straight dough method - Osmotolerance (3)

The amounts of carbon dioxide gas generated in 30%-sucrose dough and 30%-sucrose + 3 %-salt dough shown in Table 11 were measured and compared between the KKK47 strain, and commercially available baker's yeasts A, B and C.

Table 11

Dough composition

	30%-sucrose dough	30%-sucrose + 3%-salt dough
Flour	100 g	100 g
Baker's yeast	4 g	4 g
Sucrose	30 g	30 g
Salt	-	3 g
Water	52 ml	52 ml

Table 12

Amount of gas generated

	30%-sucrose dough	30%-sucrose + 3%-salt dough
KKK47	366 ml	182 ml
Commercially available baker's yeast A	322 ml	95 ml
Commercially available baker's yeast B	341 ml	98 ml
Commercially available baker's yeast C	313 ml	128 ml

In addition to 30% sucrose, salt was added to increase

osmotic pressure, and salt tolerance was examined. Among the three commercially available baker's yeast products, their ranks of fermenting ability were different between the 30%-sucrose dough and the dough further containing salt. This indicates that sucrose tolerance to up to 30% sucrose and salt tolerance (osmotolerance) are different. The KKK47 strain of the present invention has superior tolerance to osmotic pressure due to salt.

Example 6: Straight dough method - Osmotolerance (4)

The amounts of carbon dioxide gas generated in 30%-sucrose dough and 30%-sucrose + dairy product dough shown in Table 13 were measured and compared between the KKK47 strain, and commercially available baker's yeasts A, B and C.

The results are shown in Table 14.

Table 13

Dough composition

	30%-sucrose dough	30%-sucrose + dairy product dough
Flour	100 g	100 g
Baker's yeast	4 g	4 g
Sucrose	30 g	30 g
Skim milk powder	-	4 g
Milk	-	50 ml
Water	52 ml	5 ml

Table 14

Amount of gas generated

	30%-sucrose dough	30%-sucrose + dairy product dough
KKK47	355 ml	240 ml
Commercially available baker's yeast A	322 ml	180 ml
Commercially available baker's yeast B	340 ml	185 ml
Commercially available baker's yeast C	320 ml	216 ml

Fermenting ability was compared between the 30%-sucrose dough and the 30%-sucrose dough mixed with a dairy product in which dairy product tolerance (osmotolerance) was examined. The ranks of the sucrose tolerance and the fermenting ability of the three commercially available baker's yeasts were similar to those in Example 5. The KKK47 strain of the present invention is considered to be superior to the commercially available yeasts in dough which is mixed with a dairy product to increase osmotic pressure.

Example 7: Straight fermentation - Sucrose tolerance

The amounts of carbon dioxide gas generated in 30%-sucrose dough and 40%-sucrose dough shown in Table 15 were measured and compared between the KKK47 strain, and commercially available baker's yeasts A, B and C.

The results are shown in Table 16.

Table 15

Dough composition

	Amount of sucrose added	
	30%	40%
Flour	100 g	100 g
Baker's yeast	4 g	4 g
Sucrose	30 g	40 g
Water	52 ml	47 ml

Table 16

Amount of gas generated

	Amount of sucrose added	
	30%	40%
KKK47	362 ml	218 ml
Commercially available baker's yeast A	303 ml	119 ml
Commercially available baker's yeast B	346 ml	135 ml
Commercially available baker's yeast C	315 ml	174 ml

Similar to Examples 5 and 6, commercially available baker's yeast B had the highest level of fermenting ability in the 30%-sucrose dough of the three commercially available baker's yeasts. The 40%-sucrose dough resulted in different ranking of fermenting ability. In this case, commercially available baker's yeast C had the highest fermenting ability. The sucrose tolerance in the 30%-sucrose dough does not necessarily mean sucrose tolerance in dough having a sucrose concentration of at least 30%. The osmotolerant KKK47 strain has excellent fermenting ability in 40%-sucrose dough. It is therefore considered that the fermenting ability of the KKK47 strain was not much suppressed when the amount of sugar added was increased from 30% to 40%,

and that the KKK47 strain has excellent sucrose tolerance.

Example 8: Sponge dough method

Next, the amount of carbon dioxide gas generated was compared among final dough having different compositions in the sponge dough method.

Sponge dough ingredients shown in Table 17 were mixed for 3 minutes with a Hobart desk-top mixer, followed by sponge dough fermentation at 30°C for 150 minutes. The fermented sponge dough was mixed with final dough ingredients for 3 minutes with the Hobart desk-top mixer. The amount of carbon dioxide gas generated in every 50 g of the dough was measured at38°C for 2 hours using a Fermograph.

The results are shown in Table 18.

Table 17

Dough composition

	Sponge dough composition	Final dough composition		
		(1)	(2)	(3)
Flour	70 g	30 g	30 g	30 g
Baker's yeast	3 g	-	-	-
Sucrose	-	25 g	12.5 g	-
Glucose	3 g	-	6.25 g	15 g
Fructose	-	-	6.25 g	15 g
Water	39 ml	15 ml	15 ml	15 ml

Final dough composition:

(1) 25%-sucrose dough

(2) sucrose-mixed isomerized sugar containing dough having a sugar concentration of 25%

(3) isomerized sugar containing dough having a sugar concentration of 30%

Table 18

Amount of gas generated

	Final dough		
	(1)	(2)	(3)
KKK47	301 ml	264 ml	179 ml
Commercially available baker's yeast A	248 ml	198 ml	109 ml
Commercially available baker's yeast B	305 ml	235 ml	130 ml
Commercially available baker's yeast C	258 ml	219 ml	82 ml

The ranking of osmotic pressure, from lowest to highest, is (1) 25%-sucrose dough, (2)25%-sugar sucrose-mixed isomerized sugar containing dough and (3) 30%-sugar isomerized sugar containing dough. Although commercially available baker's yeast C had the greatest amount of gas generated in the high osmotic pressure dough of the straight dough method, commercially available baker's yeast B had the greatest amount of gas generated in the high osmotic pressure final dough in the sponge dough method. Different levels of osmotolerance were exhibited between the methods.

For any of the baker's yeasts, it was observed that as the osmotic pressure of dough was increased, the amount of gas generated was decreased. The KKK47 strain of the present invention generated the greatest amount of gas even in the high osmotic pressure dough, and the fermenting ability of the KKK47 strain was not much suppressed with an increase in osmotic pressure. Thus, the KKK47 strain is considered to be osmotolerant even after activation due to sponge dough

fermentation.

Example 9: Freeze tolerance

Freeze tolerance was examined.

35%-sucrose dough and sucrose-mixed isomerized sugar containing dough shown in Table 19 were prepared. The amounts of carbon dioxide gas generated in the dough were measured and compared between the KKK47 strain, and commercially available baker's yeasts A, B and C before freezing and after freezing for 4 weeks.

The results are shown in Table 20.

Table 19

Dough composition		
	35% sucrose	Sucrose-mixed isomerized sugar dough composition (30% sugar)
Flour	100 g	100 g
Baker's yeast	5 g	4 g
Sucrose	35 g	15 g
Glucose	-	7.5 g
Fructose	-	7.5 g
Salt	0.5 g	1 g
Water	50 ml	52 ml
Steps		
Mixing	Hobart desk-top mixer 3 minutes	
Dividing	A loaf of dough 50 g	
Pre-fermentation	30°C 60 minutes	
Measurement before freezing	Measure the amount of gas generated at 38°C for 2 hours	
Freezing	-30°C, 1 hour -20°C, a given time	
Thawing	25°C 1 hour	
Measuring	Measure the amount of gas generated at 38°C for 2 hours	

Table 20

Amount of gas generated before and after freezing

	35%-sucrose dough	
	Before freezing	After freezing for 4 weeks
KKK47	362 ml	351 ml
Commercially available baker's yeast B	294 ml	254 ml
Commercially available baker's yeast C	310 ml	272 ml
	30%-sucrose-mixed isomerized sugar dough	
	Before freezing	After freezing for 4 weeks
KKK47	302 ml	299 ml
Commercially available baker's yeast B	246 ml	228 ml
Commercially available baker's yeast C	287 ml	260 ml

The KKK47 strain had excellent osmotolerance. Therefore, the KKK47 strain had strong fermenting ability before freezing, and excellent freeze tolerance in sucrose-rich dough and high osmotic pressure dough, and therefore retained fermenting ability to a major extent after freeze preservation.

Example 10: Baking test with straight dough method

The KKK47 strain and commercially available baker's yeast C were subjected to a baking test with the straight dough method using a dough composition shown in Table 21, in which specific volume was measured. Specific volume was

measured with a rapeseed replacement method.

The results are shown in Table 22.

Table 21

Dough composition	
Flour	100%
Baker's yeast	3
Isomerized liquid sugar	28
Salt	1.5
Shortening	8
Yeast food	0.1
Skim milk powder	2
Hen egg	10
Water	48
Steps	
Mixing	L3M3H1 ↓ L2M2H2
Dough temperature	28°C
Floor time	90 minutes (28°C)
Dividing	400 g
Bench time	40 minutes (28°C)
Moulding	
Final proof	38°C 45 minutes
Baking	200°C 30 minutes

Table 22

Specific volume of bread

KKK47	5.2
Commercially available baker's yeast C	4.8

The KKK47 strain resulted in bread having a good specific volume in a baking test with the straight dough method using dough mixed with sucrose-mixed isomerized sugar and bread sub-ingredients such as salt, shortening, skim milk powder, etc.

Example 11: Baking test - Sponge dough (1)

The KKK47 strain and commercially available baker's yeasts B and C were subjected to a baking test with the sponge dough method using a dough composition shown in Table 23, in which specific volume was measured.

The results are shown in Table 24.

Table 23

Dough composition		
	Sponge dough	Final dough
Flour	70%	30%
Baker's yeast	3	-
Isomerized liquid sugar	3	25
Salt	-	1.5
Shortening	-	8
Yeast food	0.1	-
Skim milk powder	-	2
Hen egg	-	10
Water	40	8
Steps		
Sponge dough mixing	L3M3	
Dough temperature	24°C	
Sponge dough fermentation	28°C 2 hours	
Mixing final dough	L2M2H2 ↓ L3M3H1	
Dough temperature	28°C	
Floor time	50 minutes	
Dividing	400 g	
Bench time	15 minutes	
Final proof	38°C 45 minutes	
Baking	200°C 30 minutes	

Table 24

Specific volume of bread

KKK47	5.3
Commercially available baker's yeast B	5.0
Commercially available baker's yeast C	4.8

The final dough contained sucrose-mixed isomerized sugar which causes higher osmotic pressure than the same sugar concentration of sucrose. In this baking test using dough mixed with bread sub-ingredients, such as salt, skim milk powder, etc., the KKK47 strain resulted in bread having a larger specific volume even in the sponge dough method. Thus, it was confirmed that the KKK47 strain is suitable for breadmaking using high osmotic pressure dough.

Example 12: Sponge dough method (2)

The KKK47 strain and commercially available baker's yeasts B and C were subjected to a baking test with the sponge dough method using a dough composition shown in Table 25, in which specific volume was measured.

The results are shown in Table 26.

Table 25

Dough composition		
	Sponge dough	Final dough
Flour	70%	30%
Baker's yeast	3.5	-
Sucrose	3	35
Salt	-	1.5
Margarine	-	20
Yeast food	0.1	-
Milk	-	10
Emulsifier	0.25	-
Hen egg	-	15
Water	28	8
Steps		
Sponge dough mixing	L3M3	
Dough temperature	24°C	
Sponge dough fermentation	28°C 2 hours	
Mixing final dough	L3M5 ↓ M5 ↓ M5H1	
Dough temperature	27°C	
Floor time	28°C 60 minutes	
Dividing	400 g	
Bench time	28°C 20 minutes	
Moulding		
Final proof	38°C 50 minutes	
Baking	200°C 30 minutes	

Table 26

Specific volume of bread

KKK47	5.1
Commercially available baker's yeast B	4.4
Commercially available baker's yeast C	4.2

The KKK47 strain exhibited excellent capability for breadmaking by the sponge dough method in which the final dough composition had a sucrose concentration of as high as 35%. Thus, it was confirmed that the KKK47 strain is tolerant to high sucrose concentrations in the sponge dough method as well as the straight dough method.

Example 13: Baking test for frozen dough

The KKK47 strain and commercially available baker's yeast C were subjected to a baking test for frozen dough using a dough composition shown in Table 27, in which the specific volume was measured.

The results are shown in Table 28.

Table 27

Dough composition	
Flour (strong flour)	100%
Baker's yeast	6
Sucrose	35
Salt	1.5
Margarine	20
Dough modifier	2
Milk	10
Hen egg	15
Water	33
Steps	
Mixing	L3M5 $\downarrow$ M5 $\downarrow$ M5
Dough temperature	22°C
Floor time	28°C 30 minutes
Dividing	60 g
Bench time	15 minutes
Moulding	Roll type
Freezing	Rapid freezing at -30°C for 1 hour freeze preservation at -20°C
Thawing	25°C 60 minutes
Final proof	38°C 60 minutes
Baking	200°C 12 minutes

Table 28

Specific volume of bread

	Freeze time		
	1 week	2 weeks	4 weeks
KKK47	6.2	6.2	6.1
Commercially available baker's yeast C	5.8	5.7	5.5

This example shows freeze tolerance in the sucrose-rich dough of 35% sucrose. The KKK47 strain retained strong fermenting ability even in such sucrose-rich dough,

and excellent freeze tolerance, so that a reduction in the volume of bread due to freeze preservation was not substantially observed.

INDUSTRIAL APPLICABILITY

The baker's yeast of the present invention has excellent osmotolerance, and can be effectively used in high osmotic pressure dough for any of straight dough method bread, sponge dough method bread, and freezing method bread. Further, the baker's yeast of the present invention has excellent fermenting ability irrespective of high osmotic pressure due to isomerized sugar, salt, and other bread sub-ingredients in addition to sucrose-rich dough. Therefore, with the baker's yeast of the present invention, bread having a good volume can be produced and the range of combinations of various bread sub-ingredients can be broadened, thereby making it possible to produce a greater variety of bread products than ever before.

Various aspects of the present invention are herein described by use of the particular embodiments. Changes and modifications will be apparent from the disclosure. It will be understood that the disclosure falls within the scope and spirit of this invention as indicated in the claims appended hereto.

As used herein, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude other additives, components, integers or steps.

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction or that this prior art could reasonably be expected to be ascertained, understood and regarded as relevant by a person skilled in the art.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1 A baker's yeast having osmotolerance (as herein defined) in fermentation of bread dough, wherein the baker's yeast has osmotolerance in fermentation in a straight dough method for breadmaking, wherein the amount of carbon dioxide gas generated in a dough containing 35% isomerized sugar is at least 140 ml in every 50 g of dough, where the gas is measured at 38°C for 2 hours.

2 A baker's yeast having osmotolerance (as herein defined) in fermentation of bread dough wherein the baker's yeast has sponge dough tolerance in fermentation of final dough in a sponge dough method for breadmaking, wherein the amount of carbon dioxide gas generated in a dough containing 30% isomerized sugar is at least 140 ml in every 50 g of dough, where the gas is measured at 38°C for 2 hours.

3 A baker's yeast having osmotolerance (as herein defined) in fermentation of bread dough, wherein the amount of carbon dioxide gas generated in a dough containing 35% sugar after freezing for 4 weeks is at least 300 ml in every 50 g of dough, where the gas is measured at 38°C for 2 hours.

4 A baker's yeast according to at least two of claims 1, 2 and 3.

5 Yeast strain KKK47.

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6 Bread made using a baker's yeast according to any one of claims 1 to 5.

7 Bread dough containing a baker's yeast according to any one of claims 1 to 5.

8 A method for breadmaking, wherein a baker's yeast according to any one of claims 1 to 5 is used.

9 A method for producing baker's yeast having osmotolerance (as herein defined) in fermentation of bread dough comprising the steps of:

(a) deriving spores from yeast strains having desired characteristics,

(b) crossing spores to produce first-generation crossed strains,

(c) allowing the first-generation crossed strains to form spores,

(d) crossing spores of (c) with each other to produce second-generation crossed strains,

(e) allowing the second-generation crossed strains to form spores,

(f) crossing spores of (e) with each other to produce third-generation crossed strains.

10 A method for screening for baker's yeast having osmotolerance (as herein defined) in fermentation of bread dough comprising the steps of:

(a) mixing dough ingredients,

(b) measuring the amount of gas generated in every 50g of dough where the gas is measured at 38°C for 2 hours.

11 The method of claim 9 further comprising the screening method of claim 10 conducted after one or more of steps (b), (d) and (f).

12 A method according to any one of claims 9-11 wherein the bakers yeast is Saccharomyces cerevisiae.

13 A baker's yeast substantially as hereinbefore described with reference to any one of the examples.

14 A method according to any one of claims 7-9 substantially as hereinbefore described with reference to any one of the examples.

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